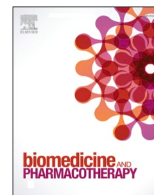
Biomedicine & Pharmacotherapy 193 (2025) 118749

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha



Review

Role of JNK3 signaling in acute ischemic stroke cerebroprotection: A systematic review

Federica Ferrari [a,b,*], Federico Mazzacane [a,b], Beatrice del Bello [a,b], Anna Cavallini [b], Domenico Raimondo [c], Tiziana Borsello [d,e,**]

[a] *Department of Brain and Behavioral Sciences, University of Pavia, Viale Golgi 19, Pavia 27100, Italy*

[b] *Department of Emergency Neurology and Stroke Unit, IRCCS Fondazione Mondino, Via Mondino 2, Pavia 27100, Italy*

[c] *Department of Molecular Medicine, Sapienza University of Rome, Laboratory Affiliated to Istituto Pasteur-Fondazione Cenci Bolognetti, Viale Regina Elena 291, Rome 00161, Italy*

[d] *Department of Pharmacological and Biomolecular Sciences, University of Milan, Via Balzaretti 9, Milan 20133, Italy*

[e] *Neuronal Death and Neuroprotection Unit, Department of Neuroscience, Mario Negri Institute for Pharmacological Research - IRCCS, Via Mario Negri 2, Milan 20156, Italy*

ARTICLE INFO

Keywords:
JNK3
Cerebroprotection
Acute ischemic stroke
Cerebral ischemia/reperfusion
Signal transduction

ABSTRACT

Ischemic stroke lacks clinically proven cerebroprotectants, partly because candidate targets have not been evaluated with rigorous translational pipelines. The aim of this study was to perform a systematic review about the potential role of the brain-enriched JNK3 signaling as a therapeutic target for acute ischemic stroke. A PRISMA-guided systematic search of PubMed, EMBASE and Scopus was performed up to October 2024. The research was limited to original research articles published in extenso on Institute for Scientific Information (ISI) Journals and written in English. Fifty-six studies met the inclusion criteria, all preclinical with a predominance for rodent models of global transient ischemia, with sparse representation of focal or large-animal models. Although the heterogeneous outcomes of the included studies, convergent evidence showed that JNK3 drives post-ischemic injury through (i) GluR6/NMDAR-PSD-95-MLK3 excitotoxic scaffolds, (ii) ASK1-initiated oxidative cascade, (iii) mitochondrial Bax/cytochrome-c apoptosis, (iv) ceramide synthesis and autophagy dysregulation, and (v) release from PI3K-AKT or HO-1 scaffold brakes. Collectively, literature supports JNK3 as a pleiotropic, druggable hub whose early blockade affords robust cerebroprotection, but translation into the clinical settings will require isoform-selective, brain-penetrant compounds evaluated in experimental conditions that more closely mirror clinical reality. Speculatively, future advances may derive from highly specific JNK3 inhibitors capable of targeting vulnerable neuronal populations within ischemic regions. When combined with optimized

Abbreviations: 2-VO, two-vessel occlusion; 4-VO, four-vessel occlusion; AKT1, protein-kinase-B isoform 1; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic-acid receptor; ASK1, apoptosis signal-regulating kinase-1; ATF2, activating transcription factor-2; Bax, Bcl-2-associated X-protein; Bcl-2, B-cell lymphoma-2; Bim, Bcl-2-interacting mediator of cell death; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; Cdc42, cell-division-cycle protein 42; CoQ_{10}, co-enzyme Q10; DNQX, 6,7-dinitroquinoxaline-2,3-dione; Dvl-1, Dishevelled-1; ERK, extracellular signal-regulated kinase; $GABA_A$/$GABA_B$, γ-aminobutyric-acid receptors A and B; GluK2/GluR6, kainate-receptor subunits 2 and 6; HO-1, heme oxygenase-1; HPK1, hematopoietic progenitor kinase-1; HSP72, 72-kDa heat-shock protein; IQ-1L/IQ-1S, lithium/sodium salts of pan-JNK inhibitor IQ-1; ISI, Institute for Scientific Information; JCR, Journal Citation Reports; JIP1, JNK-interacting protein-1; JNK3, , c-Jun N-terminal kinase 3, MAPK10); KA2, kainate-receptor subunit 2; LY294002, PI3K inhibitor; MAPK, mitogen-activated protein kinase; MKK4/7, MAPK kinases 4 and 7; MLK2/MLK3, mixed-lineage kinases 2 and 3; MTORC2, mechanistic target-of-rapamycin complex 2; NAC, N-acetyl-l-cysteine; NMDAR, N-methyl-d-aspartate receptor; NO, nitric oxide; OGD, oxygen–glucose deprivation; PDK1, 3-phosphoinositide-dependent protein kinase-1; PI3K, phosphoinositide-3-kinase; PIP_2/PIP_3, phosphatidyl-inositol-4,5-bisphosphate/-3,4,5-trisphosphate; PSD-95, postsynaptic density protein-95; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; ROS/RNS, reactive oxygen/nitrogen species; Ser674, serine-674 on MLK3; SUMO, small-ubiquitin-like modifier; SYRCLE, Systematic Review Centre for Laboratory Animal Experimentation; TAK-1, TGF-β-activated kinase-1; Tat-JBD, cell-penetrating JNK-binding-domain peptide; D-JNKI-1, retro-inverso peptide JNK inhibitor; Tpl2, tumour-progression-locus-2 kinase; WDR62, WD-repeat-containing protein 62; Wnt5a, wingless-type family member 5 A; ZnPP, zinc protoporphyrin IX.

* Corresponding author at: Department of Brain and Behavioral Sciences, University of Pavia, Viale Golgi 19, Pavia 27100, Italy.

** Corresponding author at: Department of Pharmacological and Biomolecular Sciences, University of Milan, Via Balzaretti 9, Milan 20133, Italy.

E-mail addresses: federica.ferrari03@universitadipavia.it, drfedericaferrari@gmail.com (F. Ferrari), federico.mazzacane01@universitadipavia.it (F. Mazzacane), beadtrice.delbello01@universitadipavia.it (B. del Bello), anna.cavallini@mondino.it (A. Cavallini), Domenico.raimondo@uniroma1.it (D. Raimondo), Tiziana.Borsello@unimi.it, tiziana.borsello@marionegri.it (T. Borsello).

https://doi.org/10.1016/j.biopha.2025.118749
Received 29 August 2025; Received in revised form 4 November 2025; Accepted 7 November 2025
Available online 15 November 2025
0753-3322/© 2025 The Authors. Published by Elsevier Masson SAS. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

delivery systems and personalized therapeutic strategies, such agents may ultimately contribute to redefining the cerebroprotective landscape in stroke therapy.

1. Introduction

Ischemic stroke remains a leading cause of mortality and disability, yet no cerebroprotective drug has been translated into the clinical setting despite decades of encouraging pre-clinical findings, because of many critical gaps in the bench-to-bedside translational strategy [1]. Recently, the reanalysis of the ESCAPE-NA1 and FRONTIER trials has renewed interest in Nerinetide, effective when administered within 3 h from stroke onset [2–4]. Targeting microglial metabolic reprogramming toward the M2 phenotype has also emerged as a promising anti-inflammatory mechanism [5], and human neural stem cell-derived extracellular vesicles were shown to reduce infarct volume and enhance functional recovery after ischemic stroke inhibiting neuronal apoptosis, supporting their safety and therapeutic potential for clinical translation [6]. Finally, iPSC-based organoids and CRISPR gene editing have advanced personalized testing [7]. Despite these advances, no agent has achieved consistent clinical translation, underscoring the urgent need to identify novel molecular targets and combination strategies for effective cerebroprotection.

Identifying disease-relevant molecular mechanisms and studying them with properly designed experimental research have become imperative for the evaluation and employment of next-generation cerebroprotectants. In this context, targeting the Central Nervous System (CNS) specific isoform of c-Jun N-terminal kinase (JNK), i.e. JNK3, is one of the most promising cerebroprotective strategies under study.

JNKs are part of the mitogen-activated protein kinases (MAPKs), alongside the extracellular signal regulated kinase (ERK) and the mitogen activated protein kinase p38 [8]. JNKs are activated by several stress stimuli such as oxidative stress, heat and osmotic shock, and ischemia-reperfusion insults [9–11]. Encoded in three genes, ten JNK isoforms exist: four JNK1 and four JNK2 (ubiquitous), and two JNK3 (tissue-specific) [12]. In particular regarding JNK3, transcriptomic and proteomic data have revealed that, while highly enriched throughout the CNS, JNK3 is also constitutively expressed in the testis (where it is sequestered by the testicular barrier) and at low basal levels in adult myocardium [13]. JNK3 presence and function in peripheral tissues such as the pancreas are less well-characterized but increasingly studied, especially in the context of inflammation, apoptosis, and metabolic stress [14,15].

Activation of JNKs follows the phosphorylation by the MAP 2 Kinases Kinase 3, 4 and 7 (MKK3, MKK4 and MKK7), with MKK3 as a minor contributor [16]. MKKs are in turn activated by the phosphorylation mediated by MAP3 Kinases (MAP3K or MKKKs), comprising the mixed lineage kinase 2 and 3 (MLK2, MLK3), the transforming growth factor-β-activated kinase-1 (TAK-1), the tumor progression locus-2 (Tpl2) kinase, and the apoptosis signal-regulating kinase-1 (ASK1). Equally crucial is the counter-regulation provided by MAPK phosphatases (MKPs), which dephosphorylate threonine and tyrosine residues on JNKs, thereby constraining the intensity and duration of the signal [17].

Shared MAP3Ks create inherent points of convergence between the MAPK (ERK, p38 and JNK) cascades, making stringent signal discrimination essential. Scaffold proteins confer this specificity by binding the constituent kinases of a single module, thereby accelerating phosphorylation relays while isolating them from competing pathways [18]. Paradigmatic examples of these key scaffold proteins are the JNK-interacting protein 1 (JIP1) and the β-Arrestin-2, whose importance is evident in polarized cells like neurons where they dictate compartment-restricted JNK signaling [19]. For exemple, when JNK3 is activated in the nucleus, it phosphorylates c-Jun initiating a pro-apoptotic program; conversely, post-synaptic JNK3 phosphorylates and destabilizes PSD-95, down-regulating AMPA/NMDA receptors and thereby impairing synaptic plasticity [20].

Therefore, focusing on JNK3, its activation induces compartment-specific effects, and this -in combination with its ability to phosphorylate a wide range of substrates involved in diverse cellular processes-contributes to its complex, pleiotropic functions. In fact, more than 100 JNK3 substrates have been identified including transcription factors (such as c-Jun, ATF2, NFATc2/3, ELK-1, c-Myc), cytoskeletal regulators (e.g., doublecortin, Tau, WDR62), scaffolds (JIP1/JIP3) and mitochondrial pro-apoptotic proteins [11,21,22]. The overall JNK3 signal transduction cascade is summarized in Fig. 1, together with the main JNK3-activated cellular processes both in physiological and pathological conditions. This hierarchical network identifies JNK3 as a final hub of diverse stress signals, linking upstream stress pathways to downstream processes of tissue injury and repair. It defines multiple regulatory nodes that, due to the restricted expression of JNK3 in the CNS and its specific functional roles, represent promising therapeutic targets for cerebroprotection in ischemic stroke.

The aim of this study is to evaluate the existing literature regarding JNK3 role in acute ischemic stroke for the first time through a systematic review, to provide useful information both to improve the knowledge of JNK3 potentialities as a cerebroprotective target, and to address possible gaps that could be preventing its translation into the clinical settings.

2. Materials and methods

This review is reported according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines [23].

2.1. Data sources and search

We performed a systematic literature search on Institute for Scientific Information (ISI) included Journals indexed on PubMed, Scopus, and EMBASE up to October 2024 to identify studies focused on JNK3 in acute ischemic stroke. The study has not been registered in an online deposit, but the authors assure that the search strategy and analytical approach were not altered after literature search began.

Search terms for PubMed have been: ("stroke"[MeSH Terms] OR ("cerebral ischaemia"[All Fields] OR "cerebral infarction"[MeSH Terms] OR ("cerebral"[All Fields] AND "infarction"[All Fields]) OR "cerebral infarction"[All Fields] OR ("cerebral"[All Fields] AND "ischemia"[All Fields]) OR "cerebral ischemia"[All Fields] OR "brain ischemia"[MeSH Terms] OR ("brain"[All Fields] AND "ischemia"[All Fields]) OR "brain ischemia"[All Fields]) OR ("brain ischaemia"[All Fields] OR "brain ischemia"[MeSH Terms] OR ("brain"[All Fields] AND "ischemia"[All Fields]) OR "brain ischemia"[All Fields]) OR ("brain infarction"[MeSH Terms] OR ("brain"[All Fields] AND "infarction"[All Fields]) OR "brain infarction"[All Fields]) OR ("cerebral infarction"[MeSH Terms] OR ("cerebral"[All Fields] AND "infarction"[All Fields]) OR "cerebral infarction"[All Fields])) AND ("jnk3"[All Fields] OR "jnk-3"[All Fields] OR ("janus kinase 3"[MeSH Terms] OR "janus kinase 3"[All Fields]) OR "jnk-3"[All Fields]).

2.2. Study selection

The screening of eligible publications was carried out independently by two raters (F.F. and F.M.) on the systematic review-dedicated platform PICO Portal (https://picoportal.net). First, the titles and abstracts of all citations were reviewed. Next, the full text of potentially relevant citations was reviewed. Discrepancies were resolved by discussion and consensus.

Studies were included if they met the following eligibility criteria: (1) studies focused on acute ischemic stroke; (2) studies conducted exclusively on JNK3; (3) studies with full-text available for review. Studies were excluded if they met one or more of the following criteria: (1) data published as conference proceedings and abstracts, because of the lack of sufficient experimental details to evaluate their scientific values; (2) narrative and systematic review articles; (3) article written in a language other than English.

2.3. Data extraction and quality assessment

Data was extracted from included studies on platform PICO Portal. We extracted the following data: the first author's last name, year of publication, if performed in vitro vs in vivo, on humans and/or animal models, employed animal species, experimental model of acute ischemic stroke, main findings. Data were summarized using descriptive statistics. Categorical variables were expressed as count (percentage).

Quality of studies included in the systematic review, when performed on animal models, was assessed using the 10-item SYRCLE's risk of bias tool developed on the Cochrane tool and specifically validated for animal studies [24]. Bibliographic records were organized, de-duplicated, and cited with Zotero reference manager.

3. Results

3.1. Literature search results

Following the PRISMA guidelines, the identification process began with the import of 298 records from scientific databases. Before screening, 157 records were removed due to duplicates. This left 141 records for abstract screening, where 69 were excluded through dual independent review. Subsequently, 72 reports were sought for retrieval, with 6 not retrieved, leaving 66 reports for full-text review. During this stage, 10 records were excluded for reasons including being published as conference proceedings (n = 7), and not being focused on JNK3 (n = 2); in 1 instance a paper was excluded because retracted by the Editor of the Journal where it was published. Ultimately, 56 reports were included in the final review. Study selection process is described in detail in Fig. 2, visually based on [25].

3.2. Quality of included studies

Because all the included manuscripts were preclinical studies, risk-of-bias appraisal was performed with the SYRCLE tool, as discussed in Materials and Methods section, exposing marked methodological shortcomings (Table S1). Only 2 papers provided any statement on allocation concealment. No study reported random housing of animals, i.e. if and how many animals were randomly assigned to a single cage. Blinding was also rarely reported: 2 studies declared masking of caregivers, and 2 reported blinded outcome assessment. Incomplete outcome data and selective-reporting domains were addressed in ≤ 7 % of papers.

3.3. Characteristics of included studies

Details on included studies are provided in Table S2. Of them, 11 investigations (19.6 %) were performed in vitro: 4/56 studies (7.1 %) were conducted exclusively in cell cultures, whereas 7/56 investigations

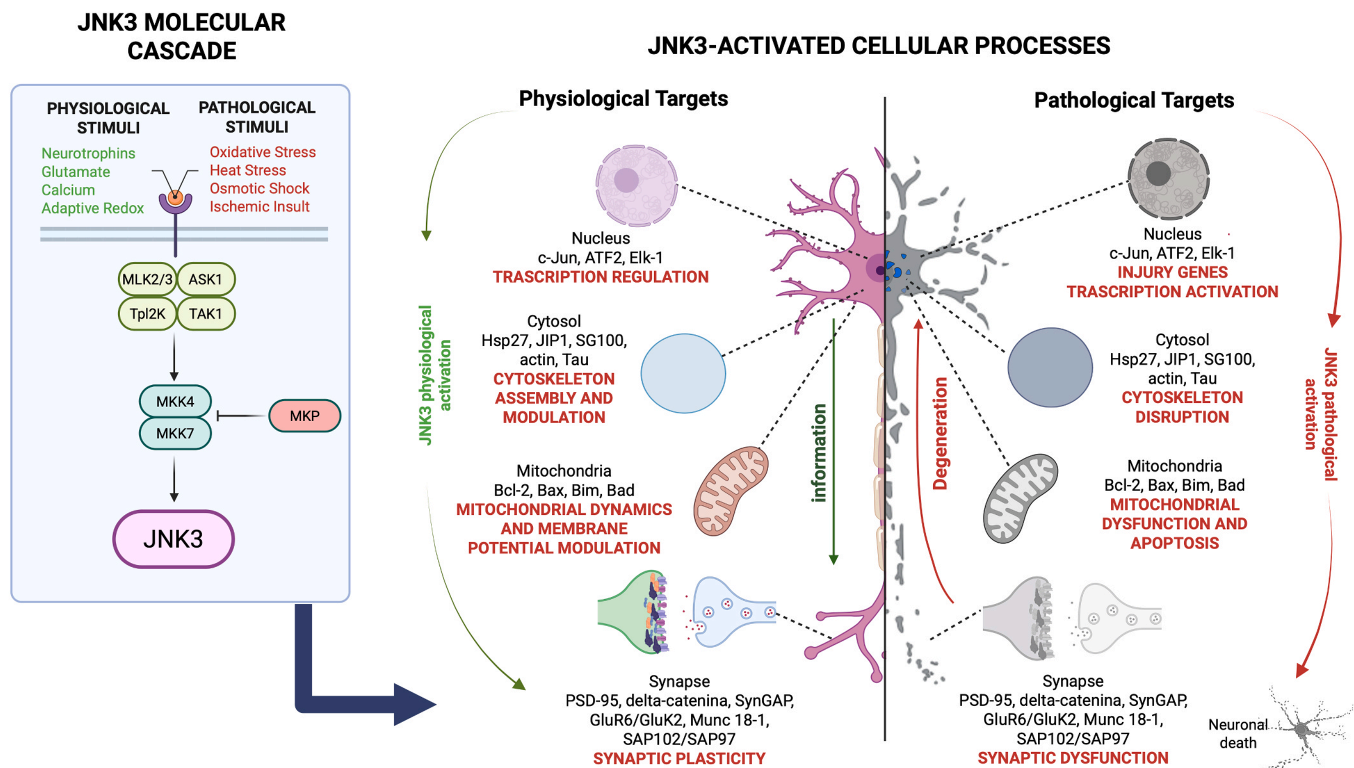


Fig. 1. JNK3 signal transduction molecular cascade (on the left) and the cellular processes activated by JNK3 in physiological and pathological conditions (on the right). Abbreviations - *Upstream kinases*: MLK2/3, Mixed Lineage Kinase 2/3; Tpl2, Tumor Progression Locus 2; ASK1, Apoptosis Signal-regulating Kinase 1; TAK1, Transforming Growth Factor Beta-Activated Kinase 1; MKK4 and MKK7, Mitogen-Activated Protein Kinase Kinase 4 and 7; MKP, MAP Kinase Phosphatase; - *Effector kinase*: JNK3, c-Jun N-terminal Kinase 3; - *JNK3 targets*: PSD-95, Postsynaptic Density Protein 95; SynGAP, Synaptic GTPase-Activating Protein; GluR6/GluK2, Glutamate Receptor 6/Kainate Receptor Subunit 2; SAP102/SAP97, Synapse-Associated Protein 102/97; AKT1, Protein Kinase B; MAP2, Microtubule-Associated Protein 2; JIPs, JNK-Interacting Proteins; Bcl-2, B-cell Lymphoma 2; Bax, Bcl-2-associated X protein; Bim, Bcl-2-interacting mediator of cell death; Bad, Bcl-2-associated Death Promoter; ELK1, ETS Like-1 Protein; ATF2, Activating Transcription Factor 2; NFATc2/3, Nuclear Factor of Activated T-cells, cytoplasmic 2/3. Created in https://BioRender.com.

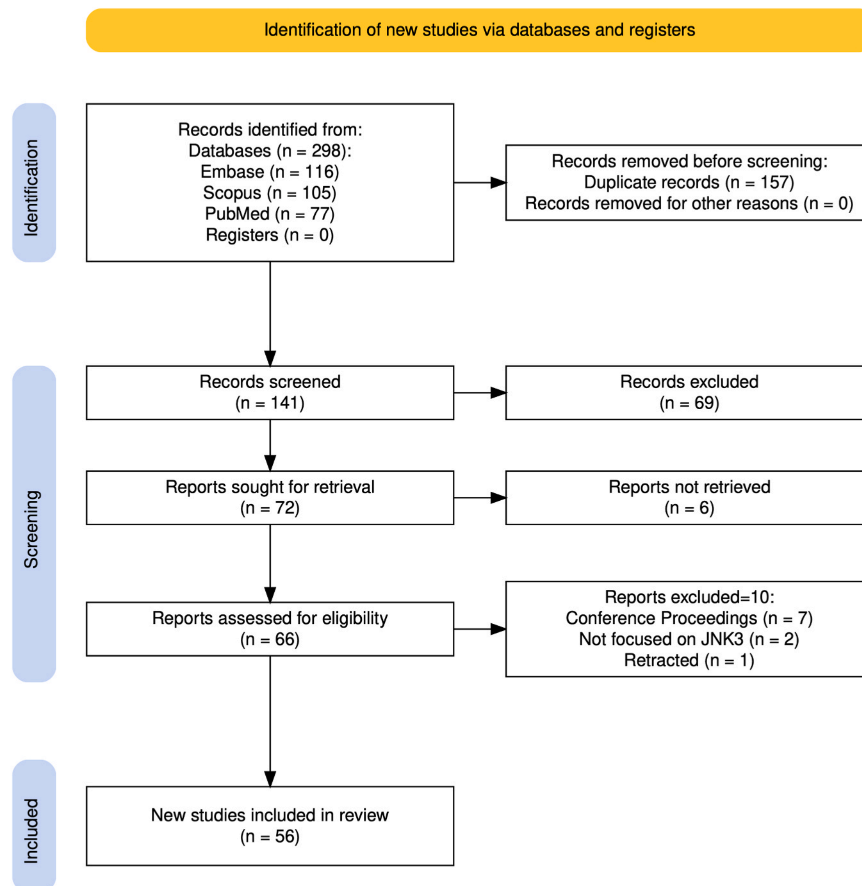


Fig. 2. PRISMA flowchart of the systematic review.

(12.5 %) combined in vitro experiments with an accompanying animal model. Among the in vitro studies, 8/11 used primary or immortalized neuronal cultures subjected to oxygen-glucose deprivation (OGD) to mimic global energy failure, 2/11 studies applied a glutamate/kainate excitotoxicity paradigm centered on the GluR6/PSD-95/MLK3 complex, and 1/11 exposed mouse HT22 neurons to a multiparametric "penumbra-mimic" medium that reproduces the acidic, hypo-nutritive milieu surrounding a focal infarct.

In vivo experiments were performed in 52 studies (92.9 %). Experimental animals were almost exclusively rodents: rats in 47/52 experiments (90.4 %) and mice in 5/52 (9.6 %). Global cerebral ischemia was the most employed modelling strategy: four-vessel occlusion (4-VO) accounted for 42/52 experiments (80.8 %), the vast majority being transient (40/42), whereas only 2 used a permanent 4-VO paradigm. Two-vessel occlusion (2-VO) was used in 2/52 studies (3.8 %), both transient with reperfusion. Focal middle cerebral artery occlusion (MCAo) provided the principal focal model, being used in 7/52 studies (13.5 %): 6/7 applied a transient protocol, and 1/7 a permanent occlusion. A single paper over 52 (1.9 %) used an alternative focal approach (transient unilateral common-carotid occlusion).

3.4. *Main physiopathological JNK3-mediated mechanisms*

As regards the evaluated physiopathological mechanisms mediated by JNK3 signaling, major findings are reported in Table 1, where all the are relative bibliographic references are reported.

3.4.1. *Receptor-coupled excitotoxic signaling*

Receptor-coupled excitotoxic signaling, typically the GluR6-NMDAR/PSD-95/MLK3 cascade, was the most common focus, appearing in 29/56 studies (52 %).

Glutamate-driven excitotoxicity recruits JNK3 in a highly ordered signaling complex involving GluK2, PSD-95 and MLK3 [26–28]. Disruption of this complex by antisense knockdowns of PSD-95 [29], peptide inhibitors that uncouple GluR6–PSD-95 binding [30], or MLK3 antagonists such as SP600125 molecule [31] or Fasudil hydrochloride [32] consistently prevents JNK3 phosphorylation and rescues vulnerable neurons. Additional regulatory mechanisms include CaMKII (whose antisense knock-down reduces GluK2 clustering and blunts JNK3 signaling) [33], and post-translational SUMOylation of GluK2 (which enhances scaffold formation under sustained glutamate exposure) [34].

However, glutamate has an ambivalent role after an ischemic insult: thanks to the increased activity of glutamate metabolizing enzymes, which utilize glutamate as a carbon donor to sustain energy-yielding cellular pathways [35], glutamate concentrations decrease within the first hour from the ischemic insult [36], and blunting it with an incorrect timing may impair the activation of synaptic plasticity. This implies that temporally tailored JNK3 inhibitors and potentially controlled-release agents may offer an optimal balance between cerebroprotection and functional recovery.

3.4.2. *Intrinsic mitochondrial apoptosis*

Intrinsic mitochondrial apoptotic pathway (Bax/Bcl-2 shift, cytochrome-c release, caspase-3 activation) was examined in 15/56 papers (27 %). Upon an ischemic insult, JNK3 emerges as a central executor of the intrinsic apoptotic pathway in the post-ischemic brain, integrating oxidative, excitotoxic and mitochondrial stress into a unified death program.

Selective JNK3 loss-of-function [13,37] or MLK3 inhibition [38] consistently blocked Bax translocation, cytochrome-c release and caspase-3 activation. Small molecules that reinforce mitochondrial integrity such as Quercetin, Metformin, Cilostazol, Atorvastatin or

Table 1
Main physiopathological processes mediated by JNK3 signaling after an acute ischemic injury.

Mechanism	N. Studies	Molecular Axis	Pathological Consequence	Tested modulator	References
Receptor-coupled excitotoxic signaling (GluR6-NMDAR/PSD-95)	29/56 (51.8 %)	Excess glutamate/kainate over-activates GluR6 or NMDARs; PSD-95 scaffolds MLK3-MKK4/7-JNK3 in the postsynaptic density; active JNK3 phosphorylates PSD-95, SynGAP and c-Jun, exacerbating Ca^{2+} influx and receptor internalization.	Acute excitotoxic neuronal loss, synaptic damage and impaired plasticity.	DNQX, NS102, Baclofen, Muscimol, Ethanol, Sevoflurane, Fasudil hydrochloride, Astragalus extract, GluR6-Tat decoy peptide, MLK3 inhibitors (SP600125, CEP−1347).	[26–34,43,50, 60,61,63,66, 68,76–87]
Intrinsic apoptosis (mitochondrial/ caspase)	15/56 (26.8 %)	Activated JNK3 translocates to mitochondria; JNK3 phosphorylates Bax and down-regulates Bcl-2, triggers mitochondrial outer-membrane permeabilization, cytochrome-c release and caspase-9/3 cascade.	Early and delayed neuronal apoptosis, expansion of infarct core and penumbra, worsened neurological outcome.	Atorvastatin, Cilostazol, Metformin, Quercetin, CoQ_{10} + Rosuvastatin, Paclitaxel, Geldanamycin, Piceatannol, Momordica-polysaccharides, nitric-oxide donors.	[37–39,44,45, 46,48,49,51, 53,55,64, 88–90]
PI3K/AKT negative regulation of JNK3	12/56 (21.4 %)	Under normal conditions AKT phosphorylates MLK3 (Ser674) to restrain MLK3–MKK4/7–JNK3; PI3K inhibition or ischemia ablates this brake, allowing robust JNK3 activation.	Loss of pro-survival signaling, promotion of apoptotic/ excitotoxic pathways.	Metformin, Cilostazol, Quercetin, Sevoflurane, NaHS, Atorvastatin, Geldanamycin; probes: LY294002 (PI3K inhibitor), PHT−427 (AKT inhibitor).	[42,43,45, 46–50,53,85, 91]
Oxidative/ ASK1-mediated stress signaling	11/56 (19.6 %)	ROS/RNS oxidize thioredoxin → release and activation of ASK1; ASK1 auto-phosphorylates, activates MKK4/7 → JNK3; downstream phosphorylation of c-Jun, Bim, Drp1 enhances oxidative injury.	Endothelial dysfunction, amplified oxidative damage, apoptotic cell loss.	NaHS (H_2S), N-acetyl-cysteine, CoPP/Hemin (HO−1 inducer), CoQ_{10} + Rosuvastatin, NO donors (e.g. SNAP), HO−1 up-regulation.	[43,44,52,53, 59,60,77,79, 81,82,92]
Autophagy/Cell survival switch	2/56 (3.6 %)	Sustained JNK3 under hypoxia-acidosis phosphorylates Bcl-2/Beclin-1, modulating Beclin-1–Vps34 complex and intersecting caspase-3 to steer autophagy versus apoptosis.	Mixed autophagic–apoptotic phenotype in peri-infarct tissue.	IQ−1S (pan-JNK with JNK3 bias); Tat-JNK3 decoy peptide (11 aa)	[54,55]
Ceramide synthase & mitochondrial dysfunction	1/56 (1.8 %)	JNK3 phosphorylates ceramide synthase-1, raising ceramide levels; ceramide destabilizes respiratory-chain complexes I/III and triggers mitochondrial permeability transition.	Mitochondrial energy failure, ATP decrease, ROS increase, necrotic/apoptotic death.	Piceatannol-derived ceramide-synthase−2 inhibitor	[56]
Scaffold modulation (JIP, β2-arrestin)	5/56 (8.9 %)	JIP1 or β-arrestin-2 assemble MLK3-MKK7-JNK3 complexes and traffic them to growth cones, synapses or nucleus; stress increases complex stability.	Compartment-specific c-Jun activation, axon retraction, synapse loss.	Tat-JIP1 peptide, Tat-GluR6 peptide, Dvl−1/β-arrestin−2 interfering peptide, HO−1 inducers (CoPP/Hemin), Sevoflurane	[42,59,61,62, 90]
Other mechanisms	6/56 (10.7 %)	Pan-JNK inhibitors (IQ-1S, IQ-1 L) or upstream Cdc42 knock-down broadly suppress MLK3-MKK7-JNK3; GABAergic potentiation (Ethanol + Muscimol) lowers network excitability and indirectly attenuates JNK3 phosphorylation.	Global suppression of stress-response genes, reduced infarct size.	IQ-1L, IQ−1S, Ethanol + Muscimol, Cdc42-siRNA, whole-brain hypothermia	[63–68]

Acronyms: AKT: also known as protein-kinase B; ASK1: apoptosis-signal regulating kinase 1; ATP: adenosine triphosphate; Bax: B-cell lymphoma-2–associated X protein; Bcl-2: B-cell lymphoma-2; Bim: Bcl-2-interacting mediator of cell death; CoPP: cobalt protoporphyrin IX; Drp1: dynamin-related protein 1); GABA: γ-aminobutyric acid; JIP: JNK-interacting protein; GluR6: kainate-type glutamate-receptor subunit 6; IQ-1L/IQ-1S: lithium and sodium salts of the pan-JNK inhibitor IQ-1; JNK3: c-Jun N-terminal kinase 3; MKK4/7: mitogen-activated protein kinase kinases 4 and 7; MLK3: mixed-lineage kinase 3; NaHS: sodium hydrosulfide; NMDAR: N-methyl-D-aspartate receptor; PI3K: phosphatidyl-inositol-3-kinase; PSD-95: postsynaptic density protein 95; ROS: reactive oxygen species; RNS: reactive nitrogen species; SynGAP: synaptic Ras-GTPase-activating protein; Vps34: class III phosphatidyl-inositol 3-kinase, vacuolar protein sorting 34.

Piceatannol proved protective, but only when their AKT- or nitric-oxide-dependent rise preceded the second, pro-apoptotic JNK3 peak at 6–24 h. Pan-JNK compounds (IQ-1L/S) and Geldanamycin widened the therapeutic window to 48 h, suggesting that multi-target JNK3 suppression may extend applicability to late-presenting stroke.

Paclitaxel and Piceatannol extend these findings by showing that down-regulation of JNK3 signaling respectively stabilizes the anti-apoptotic factor Bcl-2 [39] and improves cerebral perfusion, respectively, suggesting a synergy between anti-apoptotic support and vascular stabilization. This raises the speculative but promising idea that JNK3 modulation could have dual benefits, attenuating neuronal death while enhancing collateral blood flow, thus broadening the therapeutic utility in stroke patients with variable recanalization outcomes.

3.4.3. *PI3K/AKT-dependent negative regulation*

Twelve studies over 56 (21 %) showed that endogenous AKT directly phosphorylates MLK3 at Ser674, preventing its dimerization and blocking JNK3 signalling.

The Phosphoinositide-3-kinase (PI3K)/AKT pathway is a critical endogenous buffer that gates JNK3 activity: PI3K is a lipid kinase that converts PIP2 to PIP3 at the inner leaflet of the plasma membrane, thereby recruiting the serine/threonine kinase AKT (also known as protein-kinase-B) together with PDK1 that, with mTORC2, phosphorylates AKT [40,41]. Once activated, AKT exerts powerful pro-survival actions that directly oppose the MLK3/MKK4-MKK7/JNK3 pathway. In rat hippocampus AKT1 phosphorylates MLK3 on Ser674, preventing its dimerization and blocking downstream JNK3 signaling during the first hour of reperfusion [42,43].

Drugs that enhance PI3K/AKT1 therefore reduce JNK3 activity and limit infarct size. Quercetin heightens AKT1 phosphorylation, suppresses ASK1/JNK3 activation and curtails Bax-mediated apoptosis [44]. Also Metformin, Cilostazol and Sevoflurane augment AKT1, inhibit the JNK3-caspase-3 axis and improve cognitive outcome [45–47]. These findings support the idea that enhancing endogenous pro-survival

buffers could indirectly restrain JNK3, offering an alternative strategy for patients in whom direct kinase inhibition may pose toxicity risks. Heat-shock-protein-72 (HSP72) over-expression similarly boosts AKT1 and restrains JNK3 during global ischemia [91], whereas Atorvastatin engages an AKT1-nNOS signal that suppresses JNK3 and attenuates hippocampal injury [48]. Conversely, Geldanamycin protects by a dual mechanism: it lowers MLK3 expression and simultaneously facilitates AKT1 activation, yielding a sustained reduction in JNK3 phosphorylation [49]. Finally, positive modulation of AMPA receptors prevents GluR2 down-regulation and indirectly preserves AKT1-dependent inhibition of JNK3, illustrating receptor-level crosstalk between excitatory transmission and kinase signaling [50].

3.4.4. Oxidative/ASK1-mediated stress signalling

Eleven papers over 56 (20 %) placed attention to the redox-sensitive MAP3K ASK1 upstream of JNK3. Within minutes of reperfusion, reactive oxygen and nitrogen species oxidize thioredoxin releasing Apoptosis Signal–regulating Kinase-1 (ASK1), a redox-sensitive MAP3K that couples the burst of reactive oxygen and nitrogen species generated during reperfusion to selective JNK3 activation. The ensuing activation of the MLK3/MKK4-MKK7/JNK3 pathway promotes Bax translocation to the outer mitochondrial membrane and phosphorylation, Bim activation and Bax association, cytochrome-c release and caspase-3 activation [13].

Inhibition of this pathway has consistently been shown to preserve mitochondrial membrane potential and neuronal viability [38,51]. Pharmacological strategies that stabilize the thioredoxin-ASK1 complex, such as exogenous nitric oxide [52], CoQ_{10} co-administered with Rosuvastatin [53] or the plant flavonol Quercetin [44], attenuate ASK1 autophosphorylation, blunt downstream JNK3 signaling and shrink infarct volume by 30–60 %.

These findings support the hypothesis that targeting redox-sensing nodes upstream of JNK3 may not only provide cerebroprotection but could also serve as adjuncts to standard thrombolytic or endovascular therapies, especially in patients who present late or are ineligible for reperfusion.

3.4.5. Autophagy-apoptosis crosstalk

Two papers over 56 (3.6 %) linked sustained JNK3 activity to pathological autophagy in peri-infarct tissue. Autophagy, like apoptosis, is under control of the JNK cascade, and mounting data indicate that the JNK3 isoform is the principal switch that converts stress-induced autophagy from a transient, housekeeping response into a maladaptive death pathway after stroke [54,55]. Mechanistically, activated JNK3 phosphorylates Bcl-2 at Ser70, releasing Beclin-1 and thus removing the endogenous brake on autophagosome nucleation. Pharmacological JNK3 inhibition with IQ-1S or isoform-selective peptides restored the Bcl-2-Beclin-1 complex, normalized autophagic flux and prevents vacuolar degeneration without abolishing basal turnover [55].

3.4.6. Ceramide-induced mitochondrial dysfunction

One single paper over 56 (1.8 %) observed the ceramide-induced mitochondrial dysfunction. Multiple cellular pathways -including mitochondrial ceramide generation, cytoskeletal remodeling, and autophagy- converge on JNK3. This convergence highlights JNK3 central role as a signaling hub and suggests that its inhibition could offer broad cerebroprotective effects by simultaneously mitigating apoptotic, metabolic, and structural damage. The fact that JNK3 signaling is a highly integrated pathway is testified also by the observation that mitochondrial energy dysfunction is an early amplifier of the JNK3 death program: once activated, JNK3 translocates to the outer mitochondrial membrane where not only triggers intrinsic apoptosis, but also stimulates the activity of ceramide synthase-2, thereby raising ceramide levels that further destabilize mitochondrial integrity [56]. It should be highlighted that a Piceatannol-based inhibitor reduces ceramide accumulation and preserves mitochondrial function [57].

3.4.7. Scaffold protein-directed modulation

Five investigations over 56 (8.9 %) targeted the structural proteins that chaperone JNK3 modules. Disrupting JIP1 or β-arrestin-2 physically separated MLK3 from MKK7/JNK3 and conferred region-selective cerebroprotection even when given 1 h after reperfusion. Heme Oxygenase-1 (HO-1) is the inducible isoform of the heme-degrading enzyme which prevents toxic heme accumulation and provides antioxidant, anti-inflammatory and cytoprotective effects [58]. HO-1 displaces the JIP1 adaptor physically separating ASK1 from MKK7, thereby dampening JNK3 activity [59]. Exogenous HO-1 inducers such as Sevoflurane reproduce this action [60]. A cell-permeable peptide that competitively disrupts the scaffold GluR6-PSD-95-MLK3 attenuated JNK3 activation in CA1 pyramidal neurons and conferred robust neuroprotection even when delivered after reperfusion [61]. Beyond the synapse, β-arrestin-2 operates as an adaptor for the Wnt5a pathway: by binding Dishevelled-1 (Dvl-1) and simultaneously docking JNK3, it channels stress signals from membrane Frizzled receptors to the kinase core. Disrupting the Dvl-1-β-arrestin-2-JNK3 interaction with a designed peptide prevented c-Jun phosphorylation, preserved mitochondrial membrane potential and limited infarct volume by more than 45 % in a transient MCAo model [62]. These findings open speculative but intriguing possibilities: small molecules or peptides that mimic scaffold disruptors (e.g., interfering with GluR6/PSD-95 or Dvl-1/β-arrestin-2) could offer highly selective, non-catalytic strategies to dampen JNK3 activation without affecting its basal physiological roles. Given the role of scaffolds in spatially restricting kinase activity, these agents may confer greater regional specificity and minimize systemic toxicity, a major limitation of classical ATP-competitive inhibitors.

3.4.8. Other/multi-target mechanisms

A further 6 articles over 56 (10.7 %) reported multi-target mechanisms. Two studies used the anthrapyrazolone derivatives IQ-1L and IQ-1S, pan-JNK inhibitors with a modest preference for JNK3; administration up to 6 h after reperfusion depressed phospho-JNK3 and cleaved caspase-3, shrinking infarcts by about 40 % [63,64]. In one study, silencing the small GTPase Cdc42, that regulates cytoskeletal dynamics, dismantled the MLK3-MKK7 complex thereby suppressing JNK3, stabilizing mitochondrial membrane potential linking cytoskeletal stress to mitochondrial damage, and improving neurological scores by 40 % [65]. Two reports examined Ethanol plus Muscimol, a $GABA_A/GABA_B$ co-agonist mix that tempered early glutamate release, disrupted the GluR6/PSD-95 scaffold and transiently blunted JNK3 phosphorylation while sparing late plasticity-related oscillations [66,67]. Finally, moderate whole-brain hypothermia delivered during ischemia broadly reduced excitotoxic, oxidative and apoptotic signals, lowering JNK3 activity and infarct volume despite incomplete mechanistic dissection [68].

4. Discussion

Growing preclinical evidence indicates that the neuronal JNK3 functions as a pivotal switch between survival and death signaling after acute cerebral ischemia (see graphical abstract). For the first time, we have evaluated JNK3 potential cerebroprotective mechanisms by a systematic review, pinpointing the molecular pathways through which JNK3 inhibition could be a promising therapeutic target for ischemic stroke. A comprehensive overview of these mechanisms is reported in Fig. 3.

Overall, JNK3 emerges as a signalling hub that integrates excitotoxic, oxidative, mitochondrial and autophagic stress after brain ischemia. The diversity of successful interventions, yet the convergence on a few nodal assemblies, underscores both the vulnerability and the therapeutic promise of the kinase.

Currently, four main mechanisms of JNK3-targeted intervention emerge from the performed systematic review: (1) direct ATP-competitive kinase inhibition, (2) peptide-mediated sequestration, (3)

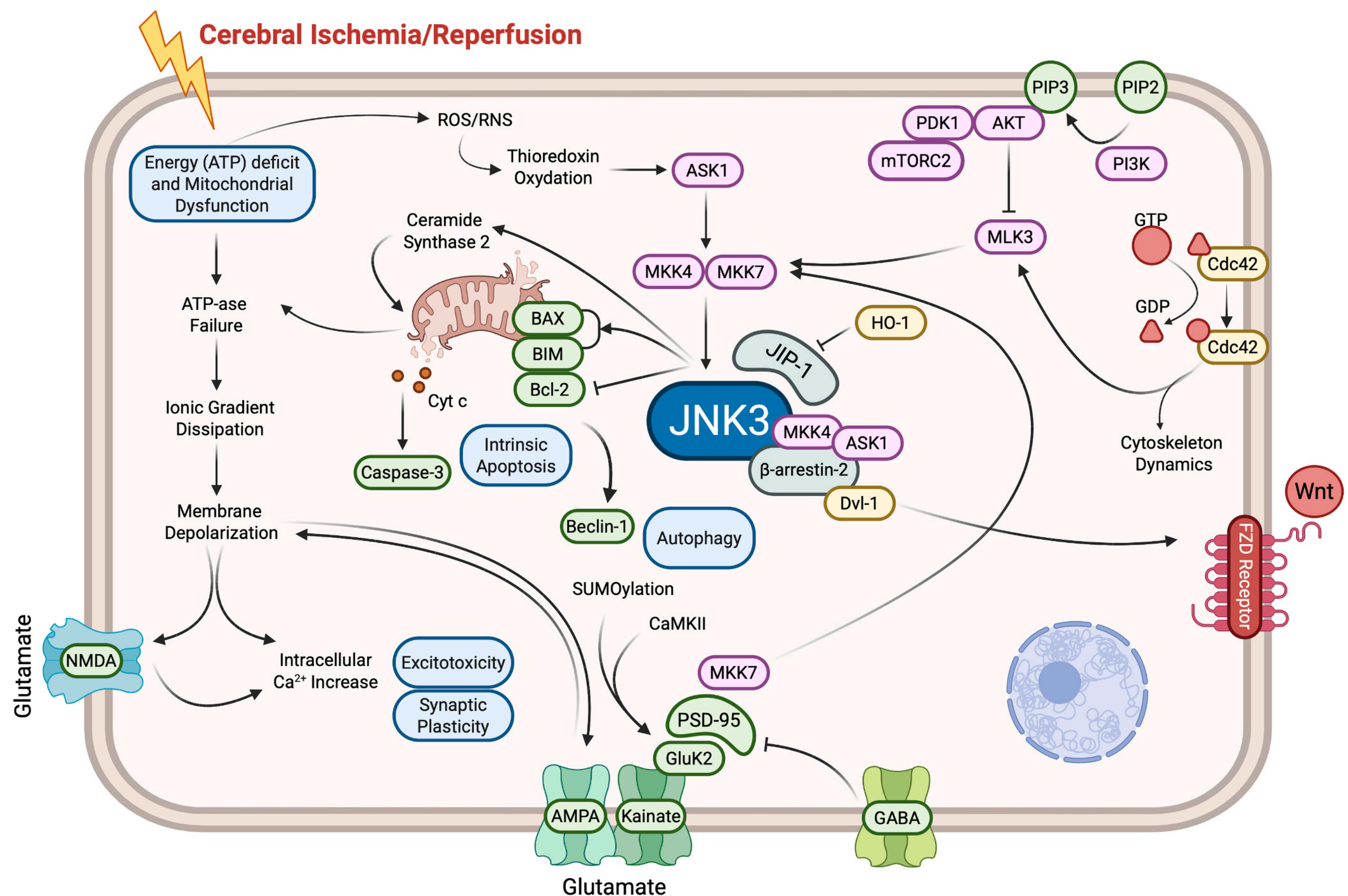


Fig. 3. Schematic representation of the main physiopathological mechanisms of c-Jun N-terminal kinase 3 (JNK3) signaling involved in cerebral ischemia and reperfusion injury. Abbreviations: ASK1, Apoptosis signal-regulating kinase 1; MKK4/7, Mitogen-activated protein kinase kinase 4/7; Cyt c, Cytochrome c; BAX, Bcl-2-associated X protein; BIM, Bcl-2-interacting mediator; Bcl-2, B-cell lymphoma 2; HO-1, Heme oxygenase 1; CaMKII, Calcium/calmodulin-dependent protein kinase II; PSD-95, Postsynaptic density protein 95; GluK2, Glutamate receptor kainate 2; NMDA, N-methyl-D-aspartate glutamatergic receptor; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid glutamatergic receptor; GABA, Gamma-aminobutyric acid receptor; Dvl-1, Dishevelled segment polarity protein 1; FZD, Frizzled Wnt receptor; PI3K, Phosphoinositide 3-kinase; PIP2/PIP3, Phosphatidylinositol 4,5-bisphosphate/Phosphatidylinositol (3,4,5)-trisphosphate; PDK1, 3-Phosphoinositide-dependent protein kinase-1; mTORC2, Mechanistic target of rapamycin complex 2; AKT, Protein kinase B; MLK3, Mixed-lineage kinase 3; SUMOylation: conjugation with small ubiquitin-like modifier; Cdc42, Cell division control protein 42; ROS/RNS. Reactive oxygen/nitrogen species. Created in https://BioRender.com.

upstream MAP3K modulation, and (4) scaffold disruption [27,31,38,62,69–71]. Adjunctive strategies such as nitric oxide donors, AKT1 enhancers, and antioxidants further expand this repertoire [47,48,52]. These are pan-JNK inhibitors, meaning they target the entire family of JNK proteins (JNK1, JNK2, and JNK3). AKT enhancers such as Atorvastatin, and antioxidants such as Butylphthalide, dampen JNK3 indirectly and broaden the therapeutic window [47,48,52]. Collectively, these multi-layered approaches highlight JNK3 "druggability" but also emphasize the need for isoform-selective, temporally controlled inhibitors that preserve the kinase late pro-repair functions while blocking its early pro-apoptotic effects. Such inhibitors are currently not available. The ideal JNK3 inhibitor may not be a "blocker" per se, but a modulator capable of distinguishing between early cytotoxic and late neuroplastic roles of the kinase. This selectivity may be achieved by targeting the toxic downstream substrates of JNK3, allowing preservation of its physiological functions while preventing pathological signaling.

Since this systematic revision was finished (October 2024), only the study by Huang et al. [72] has been published about the role of JNK3 activation in ischemic stroke cerebroprotection. In particular, using a distal MCAO mice model, they demonstrated that post-stroke white matter injury is driven by endoplasmic reticulum stress mediated by the protein kinase R-like ER kinase (PERK) in astrocytes contralateral to the ischemic lesion, which induces secretion of lipocalin-2 (LCN2), an iron-binding glycoprotein involved in neuroinflammation and cellular stress signaling. Extracellular LCN2 binds to LRP2 receptors on mature oligodendrocytes, activating the JNK3 pathway and triggering apoptosis and demyelination. Astrocyte-specific Lcn2 deletion or oligodendrocyte Lrp2 silencing preserved myelin integrity and cognitive outcomes, whereas LCN2 re-expression reversed these effects. Clinically, high serum LCN2 levels correlated with severe leukoaraiosis, identifying the astrocyte–oligodendrocyte LCN2–LRP2–JNK3 axis as a promising target for limiting secondary demyelination after ischemic stroke.

However, a major limitation is that current evidence is confined to preclinical models, predominantly rodent global ischemia. These models fail to replicate the heterogeneous presentations of focal ischemic stroke in humans. Moreover, in the majority of the included studies, ischemia was followed by reperfusion and this is not always attainable in humans, despite the increasingly frequent use of early, successfully performed acute revascularization procedures [73]. Finally, other potential factors that have not been properly addressed so far could prevent translation of these promising results into clinical setting, e.g. experiments were not performed at different animal ages, and drugs have been administered prior the induction of ischemic stroke in many instances. The reliance on pre-treatment protocols in many studies also limits translational relevance, as stroke is typically diagnosed after symptom onset.

Speculatively, the efficacy of JNK3-targeted interventions may vary dramatically across different stroke subtypes (e.g., cardioembolic vs. small vessel), patient demographics (e.g., aged vs. young brains), and comorbid states (e.g., diabetes, hypertension), areas yet to be systematically explored. By the way, converging data show that cerebroprotective interventions tested under unclear-bias conditions overestimate drug efficacy by 30–50 % and are disproportionately likely to fail in phase-II/III trials [74,75].

In conclusion, progress in stroke cerebroprotection hinges on the development of truly isoform-selective JNK3 inhibitors with brain-penetrant pharmacokinetics [93], temporal precision, and peripheral detectability. Such molecules could enable point-of-care administration during early triage or in the ambulance, potentially transforming acute stroke care. The unique characteristics of JNK3, its neuron-specific expression, integration of multiple death pathways, and assay accessibility also in peripheral fluids, make it an exceptionally attractive therapeutic target.

Beyond ischemia, JNK3 signalling intersects with neuroinflammatory and neurodegenerative cascades, raising the possibility that such inhibitors may find broader application in mixed pathology states (e.g., post-stroke dementia or vascular cognitive impairment). When combined with reperfusion therapies and metabolic support (e.g., AKT1 activators), JNK3-targeted therapy could yield synergistic protection, not just cell survival, but circuit preservation and cognitive recovery. Moreover, it would represent a paradigm shift toward molecularly targeted interventions for stroke-addressing a critical unmet need in the field.

To bridge the translational gap, future research must adopt clinically realistic models, including aged animals, comorbidity-inclusive designs, and delayed treatment protocols. The ideal development pipeline will include early biomarkers of JNK3 activation, imaging correlates, and companion diagnostics. If successful, this approach could unlock a new era of molecularly targeted stroke therapies, addressing a long-standing unmet need in neurovascular medicine.

Funding

This work was supported by the Italian Ministry of Health "Ricerca Corrente 2022–2024" granted to IRCCS Mondino Foundation (A.C.); T. B. was funded by the SEED4I and by RECOGNIZED, the European Commission's Horizon 2020 research and innovation program (No. 847749).

CRediT authorship contribution statement

Beatrice del Bello: Writing – review & editing. **Anna Cavallini:** Writing – review & editing, Funding acquisition. **Federica Ferrari:** Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Data curation, Conceptualization. **Federico Mazzacane:** Writing – review & editing, Data curation, Conceptualization. **Domenico Raimondo:** Writing – review & editing, Supervision. **Tiziana Borsello:** Writing – review & editing, Visualization, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2025.118749.

Data availability

All data have been inserted in the supplementary material file

References

[1] M. Haupt, S.T. Gerner, M. Bähr, T.R. Doeppner, Quest for quality in translational stroke research—a new dawn for neuroprotection? IJMS 23 (10) (2022 May 11) 5381.
[2] J. Christenson, M.D. Hill, R.H. Swartz, C. Adams, O. Benavente, L.K. Casaubon, et al., Efficacy and safety of intravenous nerinetide initiated by paramedics in the field for acute cerebral ischaemia within 3h of symptom onset (FRONTIER): a phase 2, multicentre, randomised, double-blind, placebo-controlled study, Lancet 405 (10478) (2025 Feb 15) 571–582.
[3] J.M. Ospel, M. Goyal, B.K. Menon, M.A. Almekhlafi, C. Zerna, R.G. Nogueira, et al., Factors influencing nerinetide effect on infarct volume in patients without alteplase in the randomized ESCAPE-NA1 trial, Stroke 56 (1) (2025 Jan) 14–21.
[4] M. Tymianski, M.D. Hill, M. Goyal, J. Christenson, B.K. Menon, R.H. Swartz, et al., Safety and efficacy of nerinetide in patients with acute ischaemic stroke enrolled in the early window: a post-hoc meta-analysis of individual patient data from three randomised trials, Lancet Neurol. 24 (3) (2025 Mar) 208–217.
[5] C. Ji, L. Sheng, K. Han, P. Yuan, W. Li, L. Chen, et al., Microglial intervention in ischemic stroke: roles and intervention strategies, Neural Regen. Res 21 (2) (2026 Feb 1) 443–454.
[6] J. Wang, M. Zhao, D. Fu, M. Wang, C. Han, Z. Lv, et al., Human neural stem cell-derived extracellular vesicles protect against ischemic stroke by activating the PI3K/AKT/mTOR pathway, Neural Regen. Res 20 (11) (2025 Nov 1) 3245–3258.
[7] P. Bindal, D. Kumar, S. Dhamnaskar, G. Kaur, Innovative approaches for the treatment of stroke: a recent update, Naunyn Schmiede Arch. Pharm. (2025 May 29).
[8] H.J. Schaeffer, M.J. Weber, Mitogen-activated protein kinases: specific messages from ubiquitous messengers, Mol. Cell Biol. 19 (4) (1999 Apr) 2435–2444.
[9] S. Armstrong, Protein kinase activation and myocardial ischemia/reperfusion injury, Cardiovasc. Res. 61 (3) (2004 Feb 15) 427–436.
[10] A.M. Bode, Z. Dong, The functional contrariety of JNK, Mol. Carcinog. 46 (8) (2007 Aug) 591–598.
[11] M.A. Bogoyevitch, B. Kobe, Uses for JNK: the Many and Varied Substrates of the c-Jun N-Terminal Kinases, Microbiol Mol. Biol. Rev. 70 (4) (2006 Dec) 1061–1095.
[12] S. Gupta, T. Barrett, A.J. Whitmarsh, J. Cavanagh, H.K. Sluss, B. Dérijard, et al., Selective interaction of JNK protein kinase isoforms with transcription factors, EMBO J. 15 (11) (1996 June 3) 2760–2770.
[13] C.Y. Kuan, A.J. Whitmarsh, D.D. Yang, G. Liao, A.J. Schloemer, C. Dong, et al., A critical role of neural-specific JNK3 for ischemic apoptosis, Proc. Natl. Acad. Sci. USA 100 (25) (2003 Dec 9) 15184–15189.
[14] B. Traub, A. Roth, M. Kornmann, U. Knippschild, J. Bischof, Stress-activated kinases as therapeutic targets in pancreatic cancer, WJG 27 (30) (2021 Aug 14) 4963–4984.
[15] S. Abdelli, J. Puyal, C. Bielmann, V. Buchillier, A. Abderrahmani, P.G.H. Clarke, et al., JNK3 is abundant in insulin-secreting cells and protects against cytokine-induced apoptosis, Diabetologia 52 (9) (2009 Sept) 1871–1880.
[16] J. Lisnock, P. Griffin, J. Calaycay, B. Frantz, J. Parsons, S.J. O'Keefe, et al., Activation of JNK3 alpha 1 requires both MKK4 and MKK7: kinetic characterization of in vitro phosphorylated JNK3 alpha 1, Biochemistry 39 (11) (2000 Mar 21) 3141–3148.
[17] X. Guo, S. An, Y. Yang, Y. Liu, Q. Hao, T. Tang, et al., Emerging role of the Jun N-terminal kinase interactome in human health, Cell Biol. Int. 42 (7) (2018 July) 756–768.
[18] C.A. Musi, G. Agrò, F. Santarella, E. Iervasi, T. Borsello, JNK3 as Therapeutic Target and Biomarker in Neurodegenerative and Neurodevelopmental Brain Diseases, Cells 9 (10) (2020 Sept 28) 2190.
[19] D.K. Morrison, R.J. Davis, Regulation of MAP kinase signaling modules by scaffold proteins in mammals, Annu Rev. Cell Dev. Biol. 19 (2003) 91–118.
[20] G.M. Thomas, D.T. Lin, M. Nuriya, R.L. Huganir, Rapid and bi-directional regulation of AMPA receptor phosphorylation and trafficking by JNK, EMBO J. 27 (2) (2008 Jan 23) 361–372.
[21] Y.T. Ip, R.J. Davis, Signal transduction by the c-Jun N-terminal kinase (JNK) — from inflammation to development, Curr. Opin. Cell Biol. 10 (2) (1998 Apr) 205–219.
[22] A. Zeke, M. Misheva, A. Reményi, M.A. Bogoyevitch, JNK signaling: regulation and functions based on complex protein-protein partnerships, Microbiol Mol. Biol. Rev. 80 (3) (2016 Sept) 793–835.
[23] D. Moher, A. Liberati, J. Tetzlaff, D.G. Altman, The PRISMA Group. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement, PLoS Med 6 (7) (2009 July 21) e1000097.
[24] C.R. Hooijmans, M.M. Rovers, R.B. De Vries, M. Leenaars, M. Ritskes-Hoitinga, M. W. Langendam, SYRCLE's risk of bias tool for animal studies, BMC Med Res Method. 14 (1) (2014 Dec) 43.
[25] N.R. Haddaway, M.J. Page, C.C. Pritchard, L.A. McGuinness, *PRISMA2020*: An R package and Shiny app for producing PRISMA 2020-compliant flow diagrams, with interactivity for optimised digital transparency and Open Synthesis, Campbell Syst. Rev. 18 (2) (2022 June) e1230.
[26] T. Li, H.M. Yu, Y.F. Sun, Y.J. Song, G.Y. Zhang, D.S. Pei, Inhibition of cerebral ischemia/reperfusion-induced injury by adenovirus expressed C-terminal amino acids of GluR6, Brain Res 1300 (2009 Dec 1) 169–176.

[27] W.W. Hu, Y. Du, C. Li, Y.J. Song, G.Y. Zhang, Neuroprotection of hypothermia against neuronal death in rat hippocampus through inhibiting the increased assembly of GluR6-PSD95-MLK3 signaling module induced by cerebral ischemia/reperfusion, Hippocampus 18 (4) (2008) 386–397.
[28] H. Tian, Q.G. Zhang, G.X. Zhu, D.S. Pei, Q.H. Guan, G.Y. Zhang, Activation of c-Jun NH2-terminal kinase 3 is mediated by the GluR6.PSD-95.MLK3 signaling module following cerebral ischemia in rat hippocampus, Brain Res 1061 (1) (2005 Nov 2) 57–66.
[29] D.S. Pei, Y.F. Sun, Q.H. Guan, Z.B. Hao, T.L. Xu, G.Y. Zhang, Postsynaptic density protein 95 antisense oligodeoxynucleotides inhibits the activation of MLK3 and JNK3 via the GluR6.PSD-95.MLK3 signaling module after transient cerebral ischemia in rat hippocampus, Neurosci. Lett. 367 (1) (2004 Aug 26) 71–75.
[30] C.Z. Yu, C. Li, D.S. Pei, Y.Y. Zong, Q. Shi, X.R. Wen, et al., Neuroprotection against transient focal cerebral ischemia and oxygen-glucose deprivation by interference with GluR6-PSD95 protein interaction, Neurochem. Res 34 (11) (2009 Nov) 2008–2021.
[31] Q.H. Guan, D.S. Pei, Q.G. Zhang, Z.B. Hao, T.L. Xu, G.Y. Zhang, The neuroprotective action of SP600125, a new inhibitor of JNK, on transient brain ischemia/reperfusion-induced neuronal death in rat hippocampal CA1 via nuclear and non-nuclear pathways, Brain Res. 1035 (1) (2005 Feb 21) 51–59.
[32] X.E. Wei, F.Y. Zhang, K. Wang, Q.X. Zhang, L.Q. Rong, Fasudil hydrochloride protects neurons in rat hippocampal CA1 region through inhibiting GluR6-MLK3-JNKs signal pathway, Cell Biochem. Biophys. 70 (1) (2014 Sept) 415–421.
[33] Z. Liu, J. Xu, X. Shen, C. Lv, T. Xu, D. Pei, CaMKII antisense oligodeoxynucleotides protect against ischemia-induced neuronal death in the rat hippocampus, J. Neurol. Sci. 314 (1–2) (2012 Mar 15) 104–110.
[34] Q.J. Zhu, Y. Xu, C.P. Du, X.Y. Hou, SUMOylation of the kainate receptor subunit GluK2 contributes to the activation of the MLK3-JNK3 pathway following kainate stimulation, FEBS Lett. 586 (9) (2012 May 7) 1259–1264.
[35] R.F. Villa, F. Ferrari, A. Gorini, Functional Proteomics Related to Energy Metabolism of Synaptosomes from Different Neuronal Systems of Rat Hippocampus during Aging, J. Proteome Res 12 (12) (2013 Dec 6) 5422–5435.
[36] F. Ferrari, A. Gorini, S. Hoyer, R.F. Villa, Glutamate metabolism in cerebral mitochondria after ischemia and post-ischemic recovery during aging: relationships with brain energy metabolism, J. Neurochem. 146 (4) (2018 Aug) 416–428.
[37] J. Zheng, J. Qi, Q. Zou, Z. Zhang, Construction of PLGA/JNK3-shRNA nanoparticles and their protective role in hippocampal neuron apoptosis induced by oxygen and glucose deprivation, RSC Adv. 8 (36) (2018 May 30) 20108–20116.
[38] J. Pan, Q.G. Zhang, G.Y. Zhang, The neuroprotective effects of K252a through inhibiting MLK3/MKK7/JNK3 signaling pathway on ischemic brain injury in rat hippocampal CA1 region, Neuroscience 131 (1) (2005) 147–159.
[39] S.H. Qi, H. Zhao, J.J. Gong, F.M. Sun, J. Yue, Q.H. Guan, et al., Neuroprotection of paclitaxel against cerebral ischemia/reperfusion-induced brain injury through JNK3 signaling pathway, J. Recept Signal Transduct. Res 31 (6) (2011 Dec) 402–407.
[40] D.R. Alessi, S.R. James, C.P. Downes, A.B. Holmes, P.R. Gaffney, C.B. Reese, et al., Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase Balpha, Curr. Biol. 7 (4) (1997 Apr 1) 261–269.
[41] D.D. Sarbassov, D.A. Guertin, S.M. Ali, D.M. Sabatini, Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex, Science 307 (5712) (2005 Feb 18) 1098–1101.
[42] J. Pan, D.S. Pei, X.H. Yin, L. Hui, G.Y. Zhang, Involvement of oxidative stress in the rapid Akt1 regulating a JNK scaffold during ischemia in rat hippocampus, Neurosci. Lett. 392 (1–2) (2006 Jan 9) 47–51.
[43] Q.G. Zhang, X.T. Wang, D. Han, X.H. Yin, G.Y. Zhang, T.L. Xu, Akt inhibits MLK3/JNK3 signaling by inactivating Rac1: a protective mechanism against ischemic brain injury, J. Neurochem. 98 (6) (2006 Sept) 1886–1898.
[44] B. Pei, M. Yang, X. Qi, X. Shen, X. Chen, F. Zhang, Quercetin ameliorates ischemia/reperfusion-induced cognitive deficits by inhibiting ASK1/JNK3/caspase-3 by enhancing the Akt signaling pathway, Biochem Biophys. Res Commun. 478 (1) (2016 Sept 9) 199–205.
[45] D.S. Qi, J.H. Tao, L.Q. Zhang, M. Li, M. Wang, R. Qu, et al., Neuroprotection of Cilostazol against ischemia/reperfusion-induced cognitive deficits through inhibiting JNK3/caspase-3 by enhancing Akt1, Brain Res 1653 (2016 Dec 15) 67–74.
[46] X.H. Ge, G.J. Zhu, D.Q. Geng, H.Z. Zhang, J.M. He, A.Z. Guo, et al., Metformin protects the brain against ischemia/reperfusion injury through PI3K/Akt1/JNK3 signaling pathways in rats, Physiol. Behav. 170 (2017 Mar 1) 115–123.
[47] X.R. Wen, Y.Y. Fu, H.Z. Liu, J. Wu, X.P. Shao, X.B. Zhang, et al., Neuroprotection of sevoflurane against ischemia/reperfusion-induced brain injury through inhibiting JNK3/Caspase-3 by enhancing Akt signaling pathway, Mol. Neurobiol. 53 (3) (2016 Apr) 1661–1671.
[48] S. Shao, M. Xu, J. Zhou, X. Ge, G. Chen, L. Guo, et al., Atorvastatin attenuates ischemia/reperfusion-induced hippocampal neurons injury via Akt-nNOS-JNK signaling pathway, Cell Mol. Neurobiol. 37 (4) (2017 May) 753–762.
[49] X.R. Wen, C. Li, Y.Y. Zong, C.Z. Yu, J. Xu, D. Han, et al., Dual inhibitory roles of geldanamycin on the c-Jun NH2-terminal kinase 3 signal pathway through suppressing the expression of mixed-lineage kinase 3 and attenuating the activation of apoptosis signal-regulating kinase 1 via facilitating the activation of Akt in ischemic brain injury, Neuroscience 156 (3) (2008 Oct 15) 483–497.
[50] Q.G. Zhang, D. Han, S.Q. Hu, C. Li, C.Z. Yu, R. Wang, et al., Positive modulation of AMPA receptors prevents downregulation of GluR2 expression and activates the Lyn-ERK1/2-CREB signaling in rat brain ischemia, Hippocampus 20 (1) (2010 Jan) 65–77.
[51] J. Gong, F. Sun, Y. Li, X. Zhou, Z. Duan, F. Duan, et al., Momordica charantia polysaccharides could protect against cerebral ischemia/reperfusion injury through inhibiting oxidative stress mediated c-Jun N-terminal kinase 3 signaling pathway, Neuropharmacology 91 (2015 Apr) 123–134.
[52] D.S. Pei, Y.J. Song, H.M. Yu, W.W. Hu, Y. Du, G.Y. Zhang, Exogenous nitric oxide negatively regulates c-Jun N-terminal kinase activation via inhibiting endogenous NO-induced S-nitrosylation during cerebral ischemia and reperfusion in rat hippocampus, J. Neurochem. 106 (4) (2008 Aug) 1952–1963.
[53] S.A. Abd El-Aal, M.A. Abd El-Fattah, H.S. El-Abhar, CoQ10 Augments Rosuvastatin Neuroprotective Effect in a Model of Global Ischemia via Inhibition of NF-κB/JNK3/Bax and Activation of Akt/FOXO3A/Bim Cues, Front Pharm. 8 (2017) 735.
[54] M.E. Pamenter, G.A. Perkins, A.K. McGinness, X.Q. Gu, M.H. Ellisman, G. G. Haddad, Autophagy and apoptosis are differentially induced in neurons and astrocytes treated with an in vitro mimic of the ischemic penumbra, PLoS One 7 (12) (2012) e51469.
[55] M.B. Plotnikov, G.A. Chernysheva, V.I. Smolyakova, O.I. Aliev, E.S. Trofimova, E. Y. Sherstoboev, et al., Neuroprotective effects of a novel inhibitor of c-jun N-terminal kinase in the rat model of transient focal cerebral ischemia, Cells 9 (8) (2020 Aug 8) 1860.
[56] J. Yu, S.A. Novgorodov, D. Chudakova, H. Zhu, A. Bielawska, J. Bielawski, et al., JNK3 signaling pathway activates ceramide synthase leading to mitochondrial dysfunction, J. Biol. Chem. 282 (35) (2007 Aug 31) 25940–25949.
[57] R.K. Rajan, M. Ramanathan, Piceatannol selectively inhibited the JNK3 enzyme and augmented apoptosis through inhibition of Bcl-2/Cyt-c/caspase-dependent pathways in the oxygen-glucose deprived SHSY-5Y cell lines: In silico and in vitro study, Chem. Biol. Drug Des. 103 (2) (2024 Feb) e14458.
[58] S.W. Ryter, J. Alam, A.M.K. Choi, Heme oxygenase-1/carbon monoxide: from basic science to therapeutic applications, Physiol. Rev. 86 (2) (2006 Apr) 583–650.
[59] Y.J. Song, C.X. Dai, M. Li, M.M. Cui, X. Ding, X.F. Zhao, et al., The potential role of HO-1 in regulating the MLK3-MKK7-JNK3 module scaffolded by JIP1 during cerebral ischemia/reperfusion in rats, Behav. Brain Res 359 (2019 Feb 1) 528–535.
[60] L. Jin, X.M. Bo, Neuroprotection of sevoflurane against ischemia/reperfusion-induced brain injury through inhibiting GluN2A/GluN2B-PSD-95-MLK3 module, Exp. Brain Res 239 (9) (2021 Sept) 2701–2709.
[61] Q.H. Guan, D.S. Pei, Y.Y. Zong, T.L. Xu, G.Y. Zhang, Neuroprotection against ischemic brain injury by a small peptide inhibitor of c-Jun N-terminal kinase (JNK) via nuclear and non-nuclear pathways, Neuroscience 139 (2) (2006 May 12) 609–627.
[62] X. Wei, J. Gong, J. Ma, T. Zhang, Y. Li, T. Lan, et al., Targeting the Dvl-1/β-arrestin2/JNK3 interaction disrupts Wnt5a-JNK3 signaling and protects hippocampal CA1 neurons during cerebral ischemia reperfusion, Neuropharmacology 135 (2018 June) 11–21.
[63] S. Brecht, R. Kirchhof, A. Chromik, M. Willesen, T. Nicolaus, G. Raivich, et al., Specific pathophysiological functions of JNK isoforms in the brain, Eur. J. Neurosci. 21 (2) (2005 Jan) 363–377.
[64] I.A. Schepetkin, G.A. Chernysheva, O.I. Aliev, L.N. Kirpotina, V.I. Smol'yakova, A. N. Osipenko, et al., Neuroprotective effects of the lithium salt of a novel JNK inhibitor in an animal model of cerebral ischemia-reperfusion, Biomedicines 10 (9) (2022 Aug 29) 2119.
[65] J. Zhao, D.S. Pei, Q.G. Zhang, G.Y. Zhang, Down-regulation Cdc42 attenuates neuronal apoptosis through inhibiting MLK3/JNK3 cascade during ischemic reperfusion in rat hippocampus, Cell Signal 19 (4) (2007 Apr) 831–843.
[66] S.H. Qi, Y. Liu, W.W. Wang, M. Wang, G.Y. Zhang, Neuroprotection of ethanol against cerebral ischemia/reperfusion induced brain injury through GABA receptor activation, Brain Res 1276 (2009 June 18) 151–158.
[67] Y. Zhao, T. Herdegen, Cerebral ischemia provokes a profound exchange of activated JNK isoforms in brain mitochondria, Mol. Cell Neurosci. 41 (2) (2009 June) 186–195.
[68] H.X. Jiang, Q.H. Guan, D.S. Pei, G.Y. Zhang, Functional cooperation between KA2 and GluR6 subunits is involved in the ischemic brain injury, J. Neurosci. Res 85 (13) (2007 Oct) 2960–2970.
[69] S. Carboni, A. Hiver, C. Szyndralewiez, P. Gaillard, J.P. Gotteland, P.A. Vitte, AS601245 (1,3-Benzothiazol-2-yl (2-{[2-(3-pyridinyl) ethyl] amino}-4 pyrimidinyl) Acetonitrile): A c-Jun NH2-Terminal Protein Kinase Inhibitor with Neuroprotective Properties, J. Pharmacol. Exp. Ther. 310 (1) (2004 July) 25–32.
[70] T. Borsello, P.G.H. Clarke, L. Hirt, A. Vercelli, M. Repici, D.F. Schorderet, et al., A peptide inhibitor of c-Jun N-terminal kinase protects against excitotoxicity and cerebral ischemia, Nat. Med 9 (9) (2003 Sept) 1180–1186.
[71] C.H. Nijboer, M.A. Van Der Kooij, F. Van Bel, F. Ohl, C.J. Heijnen, A. Kavelaars, Inhibition of the JNK/AP-1 pathway reduces neuronal death and improves behavioral outcome after neonatal hypoxic–ischemic brain injury, Brain Behav. Immun. 24 (5) (2010 July) 812–821.
[72] Z. Huang, X. Zhang, Y. Zhao, M. Zha, M. Wu, D. Wang, et al., Lipocalin-2 regulates astrocyte-oligodendrocyte interaction to drive post-stroke secondary demyelination, Cell Rep. 44 (7) (2025 July 22) 115899.
[73] S.R. Aroor, K.S. Asif, J. Potter-Vig, A. Sharma, B.K. Menon, V. Inoa, et al., Mechanical thrombectomy access for all? Challenges in increasing endovascular treatment for acute ischemic stroke in the United States, J. Stroke 24 (1) (2022 Jan) 41–48.
[74] J.A. Hirst, J. Howick, J.K. Aronson, N. Roberts, R. Perera, C. Koshiaris, et al., The need for randomization in animal trials: an overview of systematic reviews, in: B. Thombs (Ed.), PLoS ONE, 9, 2014 June 6 e98856.
[75] Sena E.S., Van Der Worp H.B., Bath P.M.W., Howells D.W., Macleod M.R. Publication Bias in Reports of Animal Stroke Studies Leads to Major Overstatement of Efficacy. Roberts I., editor. PLoS Biol. 2010 Mar 30;8(3):e1000344..

[76] Y. Du, C. Li, W.W. Hu, Y.J. Song, G.Y. Zhang, Neuroprotection of preconditioning against ischemic brain injury in rat hippocampus through inhibition of the assembly of GluR6-PSD95-mixed lineage kinase 3 signaling module via nuclear and non-nuclear pathways, Neuroscience 161 (2) (2009 June 30) 370–380.
[77] T.L. Zhang, J.L. Fu, Z. Geng, J.J. Yang, X.J. Sun, The neuroprotective effect of losartan through inhibiting AT1/ASK1/MKK4/JNK3 pathway following cerebral I/R in rat hippocampal CA1 region, CNS Neurosci. Ther. 18 (12) (2012 Dec) 981–987.
[78] Q.J. Zhu, F.S. Kong, H. Xu, Y. Wang, C.P. Du, C.C. Sun, et al., Tyrosine phosphorylation of GluK2 up-regulates kainate receptor-mediated responses and downstream signaling after brain ischemia, Proc. Natl. Acad. Sci. USA 111 (38) (2014 Sept 23) 13990–13995.
[79] H. Tian, G. Zhang, H. Li, Q. Zhang, Antioxidant NAC and AMPA/KA receptor antagonist DNQX inhibited JNK3 activation following global ischemia in rat hippocampus, Neurosci. Res 46 (2) (2003 June) 191–197.
[80] Han, D. Zhang, Q.G. Yong-Liu null, C. Li, Y.Y. Zong, C.Z. Yu, et al., Co-activation of GABA receptors inhibits the JNK3 apoptotic pathway via the disassembly of the GluR6-PSD95-MLK3 signaling module in cerebral ischemic-reperfusion, FEBS Lett. 582 (9) (2008 Apr 16) 1298–1306.
[81] T. Li, D. Han, J. Chen, X.J. Yu, G.Y. Zhang, Neuroprotection against ischemic brain injury by knockdown of hematopietic progenitor kinase 1 expression, Neuroreport 19 (6) (2008 Apr 16) 647–651.
[82] T. Li, X.J. Yu, G.Y. Zhang, Tyrosine phosphorylation of HPK1 by activated Src promotes ischemic brain injury in rat hippocampal CA1 region, FEBS Lett. 582 (13) (2008 June 11) 1894–1900.
[83] G. Liu, J. Song, Y. Guo, T. Wang, Z. Zhou, Astragalus injection protects cerebral ischemic injury by inhibiting neuronal apoptosis and the expression of JNK3 after cerebral ischemia reperfusion in rats, Behav. Brain Funct. 9 (2013 Oct 1) 36.
[84] D.A. Shackelford, R.Y. Yeh, Modulation of ERK and JNK activity by transient forebrain ischemia in rats, J. Neurosci. Res 83 (3) (2006 Feb 15) 476–488.
[85] Q. Lv, Y. Liu, D. Han, J. Xu, Y.Y. Zong, Y. Wang, et al., Neuroprotection of GluK1 kainate receptor agonist ATPA against ischemic neuronal injury through inhibiting GluK2 kainate receptor-JNK3 pathway via GABA(A) receptors, Brain Res 1456 (2012 May 25) 1–13.
[86] Q.G. Zhang, H. Tian, H.C. Li, G.Y. Zhang, Antioxidant N-acetylcysteine inhibits the activation of JNK3 mediated by the GluR6-PSD95-MLK3 signaling module during cerebral ischemia in rat hippocampus, Neurosci. Lett. 408 (3) (2006 Nov 20) 159–164.
[87] Q.G. Zhang, R.M. Wang, D. Han, L.C. Yang, J. Li, D.W. Brann, Preconditioning neuroprotection in global cerebral ischemia involves NMDA receptor-mediated ERK-JNK3 crosstalk, Neurosci. Res. 63 (3) (2009 Mar) 205–212.
[88] C.Y. Kuan, A.J. Whitmarsh, D.D. Yang, G. Liao, A.J. Schloemer, C. Dong, et al., A critical role of neural-specific JNK3 for ischemic apoptosis, Proc. Natl. Acad. Sci. USA 100 (25) (2003 Dec 9) 15184–15189.
[89] R.K. Rajan, R.P. Kumar, M. Ramanathan, Piceatannol improved cerebral blood flow and attenuated JNK3 and mitochondrial apoptotic pathway in a global ischemic model to produce neuroprotection, Naunyn Schmiede Arch. Pharm. 397 (1) (2024 Jan) 479–496.
[90] Y.J. Song, Z.M. Zong, H.Z. Liu, R. Mukasa, D.S. Pei, J. Mou, et al., Heme oxygenase-1 regulates the JNK signaling pathway through the MLK3-MKK7-JNK3 signaling module in brain ischemia injury, Brain Res. 1429 (2012 Jan 6) 1–8.
[91] D. Qi, H. Liu, J. Niu, X. Fan, X. Wen, Y. Du, et al., Heat shock protein 72 inhibits c-Jun N-terminal kinase 3 signaling pathway via Akt1 during cerebral ischemia, J. Neurol. Sci. 317 (1–2) (2012 June 15) 123–129.
[92] X. Wen, D. Qi, Y. Sun, X. Huang, F. Zhang, J. Wu, et al., H_2S attenuates cognitive deficits through Akt1/JNK3 signaling pathway in ischemic stroke, Behav. Brain Res. 269 (2014 Aug 1) 6–14.
[93] R.K. Rajan, M. Ramanathan, Identification and neuroprotective evaluation of a potential c-Jun N-terminal kinase 3 inhibitor through structure-based virtual screening and in-vitro assay, J. Comput. Aided Mol. Des. 34 (6) (2020 June) 671–682.